THE EFFECTS OF PLATELET-RICH PLASMA ON HEALING OF PARTIAL THICKNESS BURNS IN A PORCINE MODEL

EFFET DU PLASMA RICHE EN PLAQUETTES SUR LA CICATRISATION DES BRÛLURES INTERMÉDIAIRES D'UN MODÈLE PORCIN

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SUMMARY. Platelet-rich plasma (PRP) derived from autologous peripheral blood is rich in platelets that release growth factors and cytokines. We determined the effects of topically applied autologous PRP in a partial thickness porcine burn model. Partial thickness burns were created on the backs and flanks of six domestic pigs (24 burns each) using an aluminium bar preheated to 80° C for 20 seconds. After removing the necrotic epidermis, the burns were randomly treated with a topical antibiotic ointment or a single (day 2), double (days 2 and 7), or triple (days 2, 7, and 14) topical application of PRP that was prepared freshly before application. Periodic imaging and full thickness biopsies were conducted to monitor healing over 28 days. The percentage wound reepithelialization at days 11, 14, 18 and 21 did not differ significantly among the groups. By day 28 all wounds were completely (>95%) reepithelialized, and there were no differences among the groups. Time to complete healing (presented as mean, [SD]) did not differ among the groups (antibiotics, 17.1 [3.5]; single PRP, 17.6 [4.0]; double PRP, 18.4 [3.9]; and triple PRP, 17.7 [3.3] days; ANOVA P=0.43). Scar depth (presented as mean, [SD]) in mm at day 28 by treatment group was: antibiotic 5.0 [1.0], single PRP 5.5 [1.1], double PRP 5.4 [1.1], and triple PRP 5.5 [0.6], ANOVA P=0.026. We conclude that PRP results in similar rates of reepithelialization and scar depth to standard topical antibiotics in a partial thickness porcine burn model.

Keywords: partial thickness burns, platelet-rich plasma, reepithelialization, scar depth

RÉSUMÉ. *Le plasma riche en plaquettes (PRP), dérivé du sang autologue, permet le relargage de facteurs de croissance et de cytokines. Nous avons étudié l'effet de PRP appliqué localement sur un modèle de brûlure intermédiaire chez le porc. Cette brûlure du dos et des flancs était réalisée au moyen de l'application pendant 20 s d'aluminium chauffé à 80°C sur 4 groupes de 6 porcs. Après ablation de l'escarre, les animaux étaient tirés au sort pour être traités par topique antibiotique, 1 (J2), 2 (J2 et J7) ou 3 (J2, J7, J4) application locale de PRP préparé juste avant utilisation. Des photos et des biopsies ont été réalisées régulièrement pendant 28 j afin de surveiller la cicatrisation. Les pourcentages de surface cicatrisée à J11, J14, J18 et J21 étaient similaires dans tous les groupes. La cicatrisation était quasi complète (> 95%) à J28, dans tous les groupes. Le délai jusqu'à cicatrisation complète n'était pas différent dans les groupes (ANOVA, p=0,43) : Contrôle 17,1 +/- 3,5 ; PRP J2 17,6 +/- 4 ; PRP J2 et J7 18,4 +/- 3,9 ; PRP J4, J7 et J14 17,7 +/- 3,3 jours. La profondeur de la cicatrice, bien que statistiquement significative (ANOVA p = 0,026) : 5 +/- 1 (contrôle) ; 5,5 +/- 1,1 (1 PRP) ; 5,4 +/- 1,1 (2 PRP) ; 5,5 +/- 0,6 (3 PRP) mm n'est pas considérée comme cliniquement significative. PRP donne des résultats équivalents aux topiques antibiotiques sur une brûlure intermédiaire du porc.*

Mots-clés: *brûlure intermédiaire, plasma riche en plaquettes, épithélialisation, profondeur cicatricielle*

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Introduction

Of approximately 500,000 annual burns in the U.S. alone, most are superficial or partial thickness.[1] Partial thickness burns usually heal within 2-3 weeks and are generally treated with an advanced dressing or topical antibiotics. Platelet-rich plasma is derived from autologous peripheral blood in which the platelet concentration is increased 5-10 fold.[2] On contact with wounds, the platelets within the PRP are activated, releasing multiple growth factors and cytokines[3] which have been shown to accelerate healing of cutaneous and musculocutaneous illnesses and injuries.[3-8] These effects have been thought to be mediated partly through enhanced cellular proliferation, differentiation, chemotaxis and tissue morphogenesis.[3] While a limited number of studies have examined the effects of PRP on partial and full thickness burns,[9-16] the results are inconclusive.

The objective of the current study was to determine the effects of topically applied autologous PRP on healing of partial thickness porcine burns. We hypothesized that PRP would result in faster reepithelialization and reduced scar depth compared to topical antibiotics. We also wanted to determine whether there were any significant changes in platelet counts, white blood cell (WBC) counts, and growth factor concentrations in whole blood and PRP obtained from the experimental animals both before and after burn injury.

Methods

Institutional approval

Our study protocol was conducted with full approval of the Institutional Review Board (IRB) and the Division of Laboratory Animal Research (DLAR).

Animal handling, sedation and anaesthesia

Animals were fed standard pig chow and allowed to acclimate for one week. After overnight fasting, the animals were sedated with a combination of acepromazine 0.1 mg/kg, atropine 0.02 mg/kg, ketamine 20 mg/kg and xylazine 2 mg/kg by intramuscular injection. The pigs were then intubated endotracheally and maintained under a surgical plane of anaesthesia with isoflurane 0-5.0% in O_2 USP. The hair on the backs and flanks of each pig was clipped. All handling and care was in accordance with national guidelines.[17]

Experimental model

In this study we used a previously validated partial thickness porcine burn model.[16] While under general anaesthesia, burns were created using a 150 gm 2.5 cm by 2.5 cm by 7.5 cm aluminium bar. The bar was allowed to equilibrate for 5 minutes in an 80C water bath, blotted dry, and applied to the dorsum of an anaesthetized 25 kg domestic pig for 20 s with a force of 2 kg. This results in a deep partial thickness burn extending approximately halfway down into the dermis.[18] Twenty-four evenly spaced burns were created on the dorsum of six experimental animals, giving a total of 144 burns (*Fig. 1*).

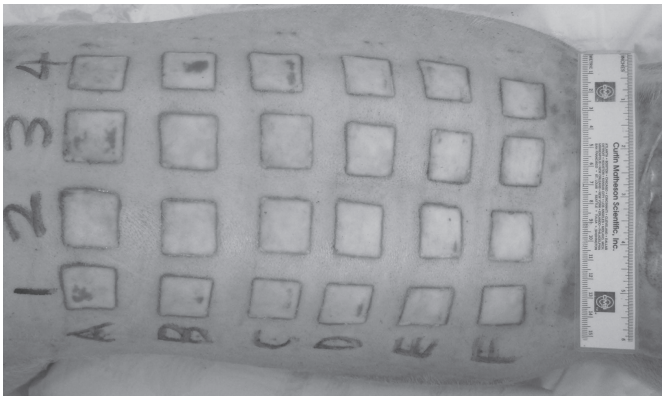

Fig. 1 - Appearance of burns two days after creation.

In order to simulate rupture and peeling of the blisters (pigs do not form blisters), the necrotic epidermis was removed immediately after injury by scraping it off with a spatula. On day 2, all wounds were surgically debrided of 0.75 mm of tissue comprising of epidermis and mid-dermis layers using an electric dermatome (Padgett Instruments, Integra Life Sciences, Plainsboro, NJ) to simulate necrotic tissue removal.

Full thickness 4 mm punch biopsies were taken at 11, 14 and 21 days post-burn from each burn, at least 10 mm from the burn edges. Full thickness 8 mm biopsies were taken from the centre of the wound at the end of the study, 28 days after injury.

Topical treatments

A preliminary study in our lab comparing a single dose of PRP (prepared freshly from peripheral blood drawn at 2, 4 or 7 days after injury) to topical antibiotics found no statistical differences in healing and scarring in the partial thickness porcine model (unpublished data). In the current study we compared the effects of 1-3 doses of PRP on healing of burns. The burns were randomly assigned to one of four treatments: (1) topical triple antibiotic ointment (Neosporin, polysporin, bacitracin) applied three times a week; (2) topical application of autologous PRP at day 2 after injury; (3) topical application of PRP at days 2 and 7 after injury; and (4) topical application of PRP at days 2, 7 and 14 after injury.

PRP preparation

Immediately prior to the application of PRP to the burn wounds, anticoagulated whole blood was drawn from the pigs' ear veins in three 60 mL sterile syringes, with each 60 mL blood comprising of 52 ml whole blood and 8 ml anticoagulant citrate dextrose formula A (ACD-A; Arteriocyte Medical Systems, Hopkinton, MA). Thus, PRP was prepared freshly, from blood collected immediately prior to the application. The blood was then processed by a commercially available device (Magellan, Arteriocyte, Hopkinton, MA) for 15 minutes to yield 7 ml of PRP from each 60 ml batch. The PRP was mixed with a combination of Ca+/thrombin in a ratio of 10:1, forming a gel in sterile 2.5 cm by 2.5 cm moulds and transferred to the wounds using a sterile spatula (*Fig. 2*). Approximately 1.5 mL of PRP+ calcified thrombin was applied to cover each wound. In order to avoid runoff of the gel-like PRP, a small amount of cyanoacrylate tissue adhesive was placed around the wound edges and immediately covered with small pieces of Tegaderm. Once the adhesive cured, the PRP remained contained within the wound under the tegaderm dressing (*Fig. 2*)

Hematology analysis was performed for each whole blood and PRP sample prepared at each designated time point to quantify the concentrations of platelets and white blood cells. Hematology analysis was performed using a Hematrue™ hematology analyzer (Heska, Loveland, CO). The Hematrue™ provided counts of platelets, and white blood cells, which is important for confirming the ability of the device to consistently concentrate these components, and to determine whether the creation of deep partial thickness burns changes the platelets and white blood cells concentration as a function of time post-burn injury.

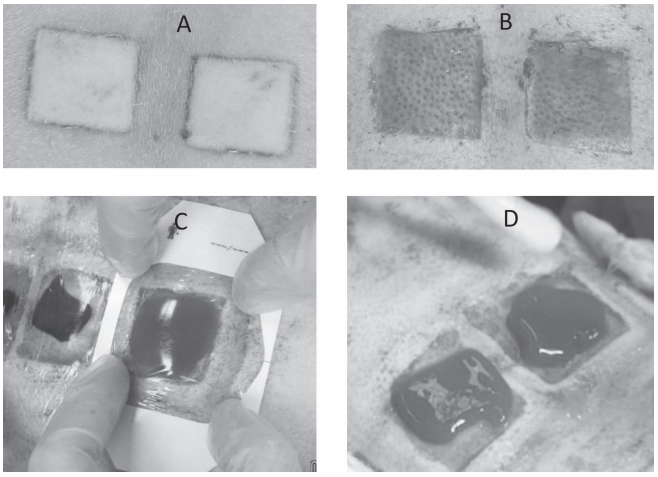

Fig. 2 - Method of applying PRP to the debrided burns. A) Appearance of non-debrided burns. B) Appearance of debrided burns. C) Application of PRP gel onto debrided burns. D) Containment of PRP gel on wound by covering it with a Tegaderm dressing that was secured to the wound edges with a topical cyanoacrylate tissue adhesive.

Dressings and topical antibiotics

All wounds were covered with non-adherent gauze (Telfa, Kendall Healthcare Products Company, Mansfield, MA). All wounds were then wrapped with a gauze bandage roll (Sof-Form, Medline Industries, Inc., Mandekein, IL), and an adhesive elastic bandage (Tensoplast, BSN Medical S.A.S., Vibraye, France). The wounds were photographed and dressings were changed three times a week for the remainder of the study, which lasted 28 days. Sedation and anaesthesia was performed as above, prior to any dressing changes or treatments.

Pain management

All animals were treated with a transdermal patch of fentanyl 50 mcg/kg every 72 hours and intramuscular buprenorphine 0.005-0.02 mg/kg every 8-12 hours as needed.

Tissue processing

The punch biopsies were bisected and fixed in 2% formaldehyde. Five-micron sections were

stained with Haematoxylin and Eosin (H&E) and imaged using an EVOS microscope (ThermoFisher Scientific) with an internal camera. Percentage reepithelialization was quantified on the tissue specimens as the linear distance of re-epithelialized burn divided by total linear distance of burn surface x 100. Depth of scarring was quantified on 28 days post-burn, bisected tissue, as the vertical distance from the epidermal-dermal junction to the dermal-subcutaneous interface. The mean scar depth for each burn was calculated from measurements of three evenly-spaced vertical distances on the two bisected halves of the wound specimens.

Determination of white blood cell and platelet counts from whole blood and PRP

In this study we used autologous PRP obtained from blood drawn at designated time points (days 2, 7, 14) immediately prior to the application of PRP to the burn wounds. In order to determine the effect of the burn injury on composition of blood and PRP, we compared platelet and white blood cell (WBC) counts in blood and PRP as a function of time. Hematology analysis was performed using a Hematrue™ analyzer to determine platelet and WBC concentrations.

Determination of growth factors in whole blood and PRP

We compared levels of four important wound healing growth factors (vascular endothelial growth factor [VEGF], platelet derived growth factor [PDGF], transforming growth factor beta 1 [TGF-B1], and basic fibroblast growth factor [FBG-basic]) released from platelets in whole blood and PRP as a function of time. Growth factors were quantified using porcine-sensitive ELISA according to the manufacturer instructions.

Data analysis

Continuous data are summarized as means and standard deviations. Binary data are summarized as counts and percentages. Differences among the groups were compared using analysis of variance (ANOVA) with the SNK post-hoc test. The significance level was set at 0.05. A sample of 25 burns in each group had 90% power to detect a 2-day difference in time to complete healing. Wounds with greater than 95% reepithelialization were considered healed.

Results

A total of 144 burns were included in the study. Of these wounds, 71 were treated with the control topical antibiotic, 25 were treated with PRP on day two, 24 were treated with PRP on days two and seven, and 24 were treated with PRP on days two, seven and fourteen. There were no wound infections or any local or systemic adverse events in any of the animals.

Wound reepithelialization and closure

The mean (SD) number of days to wound closure for the study groups were: topical antibiotics, 17.0 (3.5); PRP at day two, 17.6 (4.0); PRP at days two and seven, 18.4 (3.9), and PRP at days two, seven and fourteen, 17.8 (3.3). The differences among the groups were not statistically significant (P=0.43). The percentages for wound reepithelialization among the groups at days 11, 14, 18, 21 and 28 are presented in *Table I*. At no time point were these differences statistically significant. *Fig. 3* shows the gross appearance of the wounds at day 28, with no obvious differences among the study groups.

Fig. 3 - Gross appearance of burns 28 days after creation. There are no obvious differences between the groups.

Scar depth

The mean (SD) scar depths in mm 28 days after injury by study group were: topical antibiotics, 5.0 (1.0); PRP at day two, 5.5 (1.1); PRP at days two and seven, 5.4 (1.1); and PRP at days two, seven and fourteen, 5.5 (0.6). The difference among the groups was statistically significant (P=0.026), but unlikely to be of any clinical significance (*Fig. 4* and *Table I*).

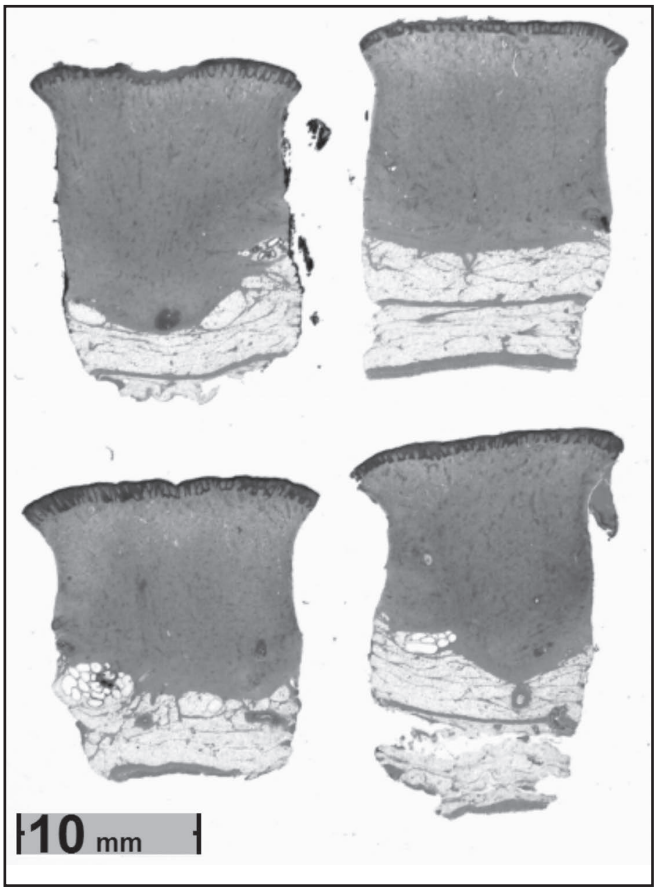

Fig. 4 - Representative 28 day micrographs of wounds treated with antibiotics (upper left), PRP at day 2 (upper right), PRP at days 2 and 7 (lower left), and PRP at days 2, 7, and 14 (lower right).

Cell counts and growth factor concentrations

There were no significant differences in the concentrations of platelets and WBCs between different time-points in whole blood or PRP. A 6-10 fold increase in platelet counts and a 2-4 fold increase in WBC counts was consistently observed in PRP compared to the whole blood at each time-point (*Fig. 5*).

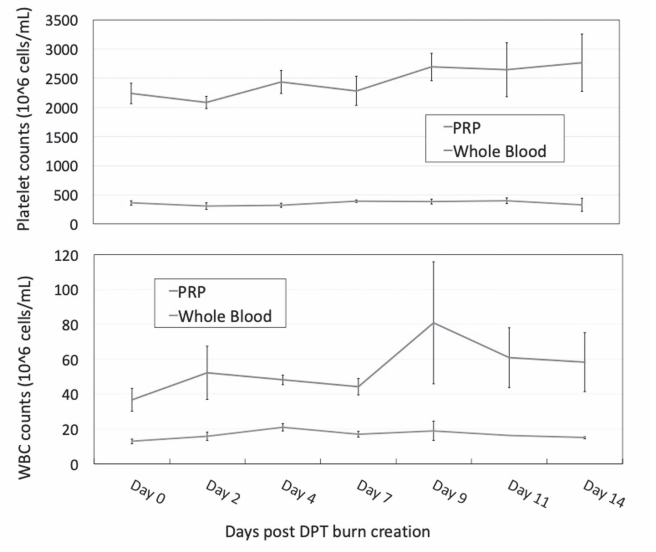

Fig. 5 - Platelet and WBC counts in whole blood and PRP.

Table I - Study outcomes

	Antibiotic	PRP day 2	PRP days 2, 7	PRP days 2, 7, 14	P value
Reepithelialization (SD), day 11, %	11 (28)	10 (26)	5 (21)	9 (21)	0.826
Reepithelialization (SD), day 14, %	47 (46)	46 (46)	32 (41)	32 (45)	0.320
Reepithelialization (SD) day 18, %	93 (21)	87 (30)	79 (37)	84 (33)	0.193
Reepithelialization (SD), day 21, %	98 (9)	100 (0)	97 (16)	100 (2)	0.569
Reepithelialization (SD), day 28, %	99 (8)	100 (0)	97 (16)	99 (10)	0.520
Time to wound closure (SD), days	17.0 (3.5)	17.6 (4.0)	18.4 (3.9)	17.8 (3.6)	0.432
Scar depth (SD), day 28, mm	5.0 (1.0)	5.6 (1.1)	5.4 (0.6)	5.2 (1.0)	0.026

PRP = platelet rich plasma

Table II - Growth factor concentrations in whole blood and PRP

		VEGF (pg/mL)	PDGF-BB (pg/mL)	TGF-β1 (pg/mL)	FGF-basic (pg/mL)
WB	**Day 0**	37.6±5.37	4063±358	9432±1021	42.8±11.2
	Day 2	41.5±7.14	3075±246	9149±3288	60.3±24.8
	Day 4	57.5±15.9	4377±750	8079±2262	67.1±26.0
	Day 7	43.2±27.1	3171±233	10832±853	58.3±26.1
	Day 9	51.0±37.2	3165±549	7229±3349	59.6±27.7
	Day 11	37.4±4.83	2609±412	9962±196	78.3±4.08
	Day 14	44.5±14.5	2037±454	8607±702	62.3±31.6
PRP	**Day 0**	76.2±24.9	10855±1146	38138±1577	261.2±69.7
	Day 2	122.1±70.5	11383±2431	39931±1860	311.1±112.9
	Day 4	84.5±25.6	12887±3114	41851±3285	376.8±119.8
	Day 7	86.1±49.2	10983±471	36651±6263	313.5±150.7
	Day 9	205.1±173.9	14180±2059	43522±3817	342.5±160.1
	Day 11	77.2±52.5	12719±1673	45303±12751	492.6±33.6
	Day 14	107.7±56.1	14494±3041	47041±6238	355.2±169.9

WB = whole blood, PRP = platelet rich plasma, VEGF = vascular endothelial growth factor, PFGF-BB = platelet derived growth factor-BB, TGF-β1 = transforming growth factor beta 1, FGF-basic = fibroblast growth factor basic.

In addition, a range of 1.5-8 fold increase in growth factor concentration in PRP over whole blood was observed, which varied depending on the particular growth factor but did not show significant differences between time-points in PRP (*Table II*). The clotting properties of whole blood and BioBandage™ did not change at any time-point.

Based on these results, it can be concluded that the concentration of platelets, growth factors and WBCs in PRP application is unlikely to alter after the creation of deep partial thickness burns in the porcine model.

Discussion

Platelets are a source of numerous growth factors and cytokines, including platelet-derived growth factor (PDGF), transforming growth factor- (TGF-), fibroblast growth factor (FGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and connective tissue growth factor (CTGF).[3] As a result, platelets play an essential role in all phases of wound healing.[19] Platelet-rich plasma has been explored for both acute and chronic wounds in numerous clinical settings, including plastic and aesthetic surgery, orthopaedics, dentistry, dermatology and cardiothoracic surgery.[4-8] While a number of studies have evaluated the use of PRP for burns, its role in this context remains unclear.[9-16]

Our results demonstrate that platelet-rich plasma administered once (at day 2), twice (at days 2 and 7), or three times (at days 2, 7 and 14) resulted in similar times to complete wound closure, or percentage reepithelialization at days 14-21 to topical antibiotics in a partial thickness porcine model. Compared with standard topical antibiotics, application of PRP also resulted in essentially similar scar depth. These findings are in contrast to other studies suggesting beneficial effects of PRP on the healing of burns. Klosova et al. studied the mechanical properties of 38 scars on 23 patients with deep burns that were treated with PRP in combination with a split thickness skin graft.[13] They found that the addition of PRP resulted in more rapid return of the viscoelastic properties of the scars to normal. A randomized controlled trial by Marck et al. of 52 patients with deep dermal burns suggested that PRP-treated areas had better or equal reepithelialization rates at 5-7 days.[12] However, no differences were noted with regards to graft take and

scar quality. A study by Venter et al. found that PRP accelerated the healing of deep second degree burns in normal and diabetic rats but not in normal rats with third degree burns.[14] Ozcelik et al. compared the effects of PRP and a control treatment on partial thickness burns in rats for up to seven days after injury. They found higher hydroxyproline levels and less inflammatory cells in burns treated with PRP. However, there were no significant differences between the groups in fibroblast development, collagen production, vessel proliferation or epithelialization. Our group also studied the effects of PRP in a full thickness porcine burn model in which some of the wounds were excised and autografted, and failed to demonstrate any difference between burns treated with PRP or a topical antibiotic.[16]

We demonstrated that WBC and platelet counts did not change significantly over time after burn injury. In addition, concentrations of growth factors thought to play a significant role in wound healing remained stable both in whole blood and in PRP obtained from animals after burn injury. These findings justify the use of autologous PRP that was obtained from the animals prior to burn injury, while this would not be realistic in the clinical scenario.

It is unclear why PRP did not improve healing and reduce scarring when compared to standard topical antibiotics in our study. It is possible that the use of an absorbent dressing on top of the PRP treatment resulted in the removal of the bioactive factors from the wound bed, wound bed desiccation, and the formation of a burn eschar, which served as a further barrier to permeation of the growth factors released by the PRP, preventing their activity in repeated dosing. Another possible explanation for the lack of benefit of PRP is that it may have caused excessive inflammation, negating its other potentially beneficial effects. We also may have chosen the wrong timing and dosing of PRP. Of note, our results in pigs may not be translatable to human burns. Importantly, wound healing is dynamic and complex, involving many cells and wound healing mediators, and not just platelets. As a result, platelet lysate alone does not have a major impact on healing. Finally, the lack of a negative control group receiving no treatment at all is a limitation. However, we felt that the topical antibiotic ointment was considered as standard care, thus no negative control was necessary. A prior study in pigs demonstrated superiority of the topical antibiotic compared with an advanced polyurethane foam dressing impregnated with silver.[20]

Conclusions

Our results demonstrate that following the timing and dosing that we applied in our study, PRP showed similar rates of reepithelialization and scar depth to a topically applied, petrolatum-based triple antibiotic ointment in a porcine partial thickness burn model. We further suggest the use of an occlusive and non-absorbing dressing for the duration of PRP applications on burn wounds in any future studies.

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Conflict of interest: None

E R R A T A C O R R I G E

We apologize for an error concerning the article
"Infection Control in German-Speaking Burn Centers: Results of an online Survey"
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1) The correct affiliation of H-O Rennekampff is
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